Protocol for double digestion (50μl system)

Pipette the following into a 1.5ml microfuge tube:

Enzyme A	2μl
Enzyme B	2μl
10× buffer	5μl
DNA	0.5-1μg
dd H_2O	rest of the volume

incubate at recommended temperature (37℃) for at least 2 hour;

Purify the digestion product;

Notes:

The enzymes used here are TaKaRa enzymes (*Eco*RI/ *Xba*I/ *Spe*I/ *Pst*I), and buffer M or H is suitable for most of double digestion;

For 20μl reaction system, the suggested amount of each restriction enzyme is 1μl;